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PLATELETS IN RAT ANAPHYLAXIS

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The anaphylactic alterations of the haemostatic mechanisms, depending upon the species and the severity of the episode, may play an important part in the pathomechanism of anaphylactic shock (14). We have previously shown that in the anaphylactic shock of rats, the fibrinolytic system became highly activated (6) and as a result *in vivo* fibrinogen degradation took place (5). In a further paper it was also shown, that during rat anaphylaxis, activation and consumption of the Hageman factor took place; and how, in the final outcome, an intimate regulatory interaction of altered haemostatic processes may result, i.e., in the prevalence of kinin generation (3). The aim of the present study was to investigate the role of platelets in the anaphylactic disturbance of haemostasis as well as in the pathomechanism of anaphylactic symptoms.

MATERIALS AND METHODS

Wistar male rats (150-200 g) were sensitized by an intraperitoneal injection of 1 ml horse serum (HS) and 3×10^{10} organisms of *Bordetella pertussis* vaccine (BPV). Control animals (CA) received only BPV. Anaphylactic shock (AS) was induced 12 days later by 1 ml HS administered intravenously during deep pentobarbital anaesthesia (40 mg/kg pentobarbital i.p. 45 min previously). Control rats were also given 1 ml HS.

Counting of platelets. At various intervals, following the challenge of anaphylaxis, blood was obtained with a plastic syringe from the abdominal aorta, and platelets were enumerated by phase contrast microscopy (17). In some experiments rats were pretreated with 100 mg/kg heparin (i.v.) 30 min before anaphylaxis, or with 10 mg/kg dipyridamole (Persantin, gift of

Boehringer Ingelheim) (i.v.) 5 min before anaphylaxis. Controls were given an equal volume (1 ml) of saline.

Radioisotope experiments. Platelet rich plasma (PRP) was prepared from the citrated (1:10 volume of 3.8 per cent Na-citrate) blood of sensitized rats in siliconized tubes by centrifugation at 290 g for 15 min. The platelets were labelled with ^{51}Cr by the method of Abrahamsen (1). ^{51}Cr labelled platelets were reinjected into sensitized rats (5×10^8 platelets into each animal) 1 h before saline (CA) or HS (AS) injection. Ten minutes following anaphylactic challenge or saline injection, arterial blood samples were obtained and the animals were killed. The radioactivity and the haemoglobin content of various organs were measured. The c.p.m. values were corrected for the blood content of various organs (18).

In vitro tests of platelet functions. Arterial blood samples of control and anaphylactized animals were obtained 5 min following saline (CA) or HS (AS) injections and PRP or platelet poor plasma (PPP, centrifugation at 2,000 g for 15 min) was prepared from them. PRP, in which the number of platelets was made constant by the addition of PPP, was used to test the following platelet functions: 1. Clot retraction, 0.2 ml of CaCl_2 was added to 0.4 ml of PRP at 37°C in triplicate. The mixture was rapidly stirred with a glass rod, then left inside the tube. Thirty minutes later the rod to which the clot was attached was gently removed and the volume of the serum expressed was measured by a Hamilton Microliter syringe. The measure of clot retraction was quantitated by relating the volume of extruded serum to the total volume of the mixture (7). 2. Platelet activity ratio (PF_3 availability) was assayed according to Saleem et al. (15). 3. Platelet adhesiveness was estimated by Salzman's technique (16). 4. Serotonin uptake and release was followed by the method of Massini & Lüscher (10).

Rat tail bleeding time was measured according to the method of Nordoy & Chandler (13).

Statistics. Mean values $\pm$ S.E. are indicated. Significance was assessed by Student's *t* test.

RESULTS

In the first 2 min following anaphylactic challenge the number of circulating platelets dropped abruptly and then decreased moderately (Fig. 1). Heparin pretreatment could not prevent the disappearance of platelets from the circulation (Table 1). In contrast, the decrease of platelet count was significantly inhibited in Persantin-treated anaphylactic animals (Table 1).

One hour after the injection of ^{51}Cr platelets into BPV and HS pretreated animals, almost 75 per cent of added radio-

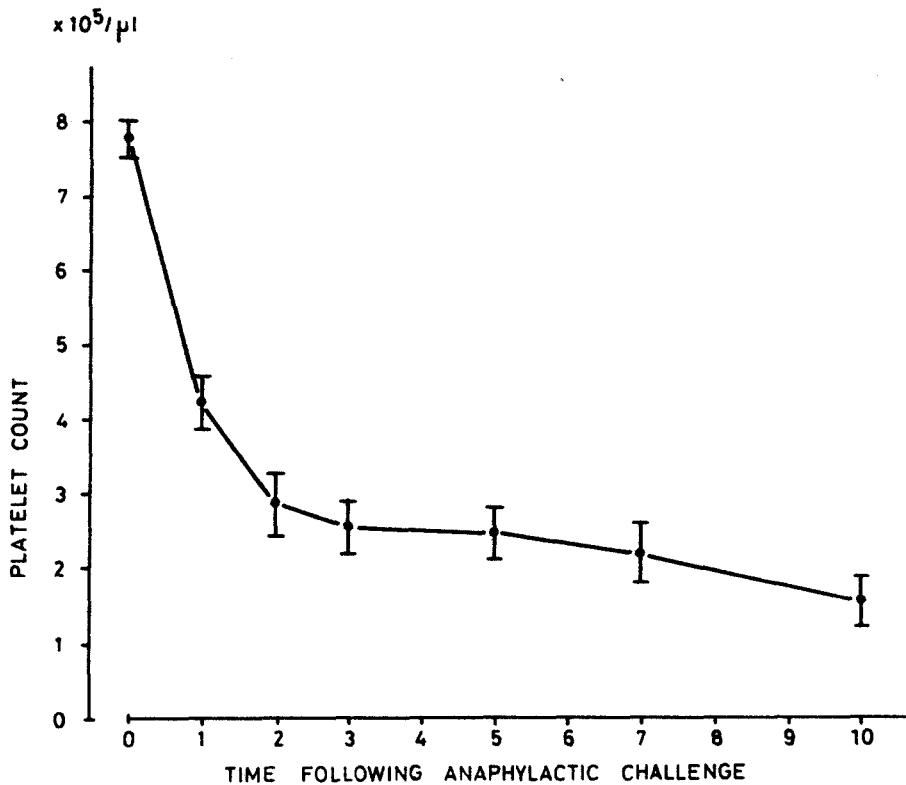


Fig. 1.

The number of circulating platelets at various intervals following anaphylactic challenge. Each point represents eight experiments.

activity was connected to circulating platelets, and the spleen, liver and lung contained the remainder (Fig. 2). When these animals were submitted to anaphylactic shock 10 min after the antigen injection, only 25 per cent of the radioactivity was connected to circulating platelets, and highly increased c.p.m. values were measured in the spleen. The ⁵¹Cr radioactivity of the lung and the small intestine also significantly increased and a certain amount could also be detected in the PPP. The distribution of radioactivity in the animals submitted to AS did not change when heparin was added before AS (not included in the figures).

Comparing their functions (clot retraction, PF₃ availability, adhesiveness, serotonin uptake and release) there was no difference between control platelets and those remaining in the circulation 5 min after anaphylactic challenge (Table 2).

TABLE 1.

The Effect of Heparin and Dipyridamole Pretreatment on the Number of Circulating Platelets Counted 5 Minutes Following HS Injections.

Groups	Pretreatment	Platelet count $\times 10^5/\mu\text{l}$	P
CA	Saline	7.2 ± 0.6	
CA	Heparin	7.3 ± 0.8	—
CA	Persantin	6.9 ± 0.9	—
AS	Saline	2.6 ± 1.25	< 0.001
AS	Heparin	2.8 ± 1.0	< 0.001
AS	Persantin	5.3 ± 1.3	< 0.001

Eight animals in each group.

TABLE 2.

Results of Functional Tests of Platelets Derived from Control and Anaphylactized Animals.

Test	CA	AS
Clot retraction (retracted volume)	$89 \pm 5 \%$	$84 \pm 9 \%$
* PF ₃ availability (platelet activity ratio)	1.45 ± 1.64	1.42 ± 2.1
* Adhesiveness	$62 \pm 15 \%$	$58 \pm 18 \%$
* Serotonin uptake	95 %	95 %
* Serotonin release (using 0.5 h/ml thrombin)	$38 \pm 4 \%$	$35 \pm 5 \%$

Eight animals in each group.

* Platelet count: 200,000/ μl in each case (diluted by PPP).

TABLE 3.

Rat Tail Bleeding Times 5 Minutes Following HS Injection.

Groups	Pretreatment	Bleeding times (sec)	P
CA	Saline	75 ± 12	
CA	Persantin	78 ± 10	—
AS	Saline	148 ± 20	< 0.001
AS	Persantin	82 ± 13	—

Eight animals in each group.

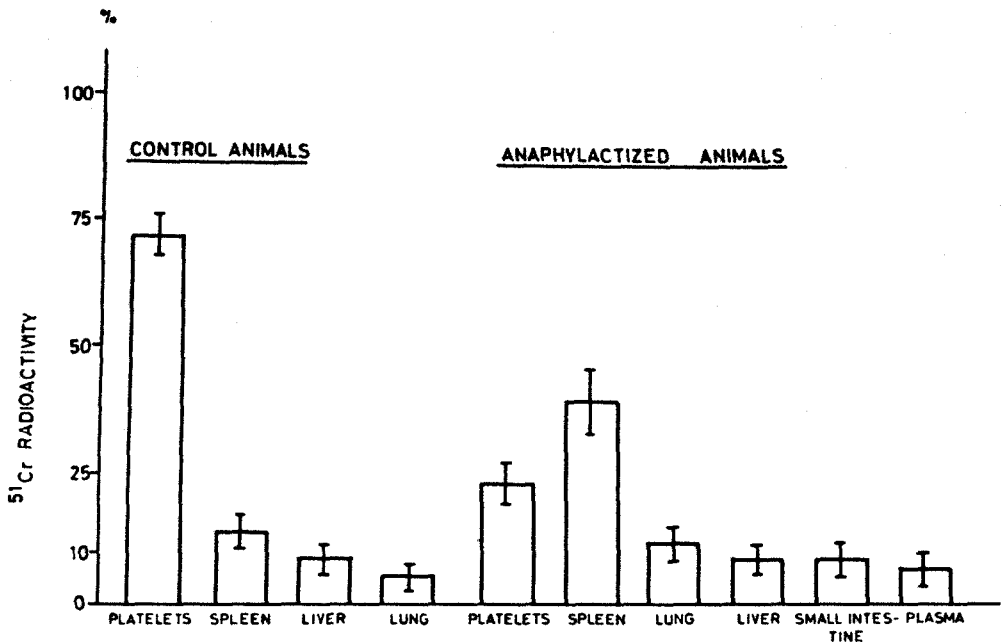


Fig. 2.

The organ distribution of ^{51}Cr radioactivity in control and anaphylactized animals. Ten animals in each group. We could not detect radioactivity in the PPP and small intestine of control animals. Significance values in case of platelets, spleen, small intestine and plasma: $P < 0.001$; lung: $P < 0.01$.

On the other hand, the rat tail bleeding time was significantly lengthened in anaphylactic rats and its prolongation was prevented by Persantin pretreatment (Table 3).

DISCUSSION

A prompt and dramatic drop of platelet count could be observed in the very early stage of rat anaphylaxis. The formation of platelet aggregating antigen-antibody complexes may be a logical explanation of this phenomenon (11). However, in *in vitro* conditions platelet aggregation was not elicited by the addition of antigen to PRP of sensitized animals (4). Furthermore, the anaphylactic shock of pertussis vaccinated rats is mediated through the reaction of antigen with cell bound IgE but not by immunocomplexes (8, 20). Thrombinaemia, as a result of the anaphylactic activation of intrinsic coagulation (3), may also aggregate circulating platelets. The

failure of heparin treatment to prevent the drop in platelet number and the fact that no systemic intravascular coagulation occurs in rat anaphylaxis (3) exclude this possibility, too.

Dipyridamole, a special inhibitor of platelet aggregation (12), could inhibit the disappearance of platelets from the circulation, supporting the idea that an aggregating agent released from immunologic target cells plays a crucial role in this phenomenon. On the basis of our present knowledge the most probable aggregating agent seems to be the so-called "platelet activating factor" which is released from sensitized human basophils (2) and rat mast cells (9) by specific antigen. The general effects of platelet activating factor and its role in the rapid drop of platelet count in anaphylactic shock are under investigation.

Rat tail bleeding time, a common *in vivo* test of the haemostatic functions of platelets, was significantly lengthened in anaphylactic shock. The reason for this phenomenon could be the decreased number of platelets in the circulation and/or that the remaining platelets are also damaged and cannot fulfil their haemostatic functions. Those platelets which remained in the circulation retained their functional integrity as was clearly proved by investigating their ability to retract the clot, to adhere to artificial surfaces, to provide PF_3 , to uptake serotonin and to release it following the addition of thrombin. In our previous study it was also demonstrated that the decreased ADP, thrombin and collagen-induced platelet aggregation observed in anaphylactic PRP was due to the anaphylactic activation of the Hageman factor but not to platelet damage (4). When the anaphylactic decrease of platelet count was prevented by Persantin, the tail bleeding time was not lengthened. Consequently, its lengthening is a consequence of the decreased number of platelets alone.

Most disappeared platelet radioactivity was found in the spleen, which probably sifts out the aggregated platelets from the circulation during AS. The radioactivity of the small intestine and the lung—the shock organs in the rat—was also increased, which means that platelets or platelet aggregates

were attached to or trapped in the damaged small vessels of these organs. This increase is not due to local blood coagulation since in heparinized rats the same increase was measured. The occlusion of the small vessels by platelet aggregates, especially in the lung and in the small intestine, may be an important factor in the development of severe shock symptoms.

The above results clearly show that platelets are involved in the pathomechanism of anaphylactic shock. Their participation in the development of shock symptoms may be summarized as follows:

1. Source of released shock mediators (11, 19).
2. Involvement in haemostatic disturbances.
3. Occlusion of small vessels by platelet aggregates.

SUMMARY

The number of circulating platelets dropped abruptly in the early phase of severe anaphylactic shock (AS) of the rat and could be inhibited by Persantin but not by heparin pretreatment. The results strongly suggest that an aggregating agent, perhaps "platelet aggregating factor", formed or released during anaphylaxis is responsible for the decrease of platelet number. The organ distribution of ^{51}Cr labelled platelets showed that in AS the aggregated platelets were removed from the circulation mostly by the spleen and part of them were trapped in the lung and the small intestine. The remaining platelets retained their functional integrity. The loss of circulating platelets is manifested by the prolongation of rat tail bleeding time and is one of the factors participating in the haemostatic disturbances during anaphylaxis.

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